Fructose modulates GLUT5 mRNA stability in differentiated Caco-2 cells: role of cAMP-signalling pathway and PABP (polyadenylated-binding protein)-interacting protein (Paip) 2

Florence GOUYON*, Cercina ONESTO†, Veronique DALET*, Gilles PAGES†, Armelle LETURQUE* and Edith BROT-LAROCHE*[1]

*Institut National de la Santé et de la Recherche Médicale U505, Université Pierre et Marie Curie, 15 rue de l'Ecole de Médecine, 75006 Paris, France, and †Université de Nice-Sophia Antipolis, Centre National de la Recherche Scientifique-Unité Mixtie de Recherche 6543, Centre A. Lacassagne, 33 avenue de Valombrose, 06189 Nice, Cedex 2, France

In intestinal cells, levels of the fructose transporter GLUT5 are increased by glucose and to a greater extent by fructose. We investigated the mechanism by which fructose increases GLUT5 expression. In Caco-2 cells, fructose and glucose increased activity of the $-2500/+41$ GLUT5 promoter to the same extent. cAMP also activated the GLUT5 promoter. However, if a protein kinase A inhibitor was used to block cAMP signalling, extensive GLUT5 mRNA degradation was observed, with no change in basal transcription levels demonstrating the involvement of cAMP in GLUT5 mRNA stability. Indeed, the half-life of GLUT5 mRNA was correlated ($R^2 = 0.9913$) with cellular cAMP levels. Fructose increased cAMP concentration more than glucose, accounting for the stronger effect of fructose when compared with that of glucose on GLUT5 production. We identified several complexes between GLUT5 3′-UTR RNA (where UTR stands for untranslated region) and cytosolic proteins that might participate in mRNA processing. Strong binding of a 140 kDa complex I was observed in sugar-deprived cells, with levels of binding lower in the presence of fructose and glucose by factors of 12 and 6 respectively. This may account for differences in the effects of fructose and glucose. In contrast, the amounts of two complexes of 96 and 48 kDa increased equally after stimulation with either glucose or fructose. Finally, PABP (polyadenylated-binding protein)-interacting protein 2, a destabilizing partner of PABP, was identified as a component of GLUT5 3′-UTR RNA–protein complexes. We conclude that the post-transcriptional regulation of GLUT5 by fructose involves increases in mRNA stability mediated by the cAMP pathway and Paip2 (PABP-interacting protein 2) binding.

Key words: Caco-2/TC7, fructose, glucose, intestine, protein kinase A (PKA), SLC2A5.

INTRODUCTION

Intestinal fructose absorption at the brush-border membrane involves GLUT5 transporter activity. GLUT5 (encoded by the *SLC2A5* gene), a member of the facilitative sugar transporter family, is present mostly in the small intestine and in the proximal tubule of the kidney [1].

Fructose stimulates GLUT5 expression to a greater extent than glucose, both *in vivo* [2,3] and in Caco-2 cells [4]. The mechanism underlying the specific regulation by fructose is largely unknown. Transcriptional [5] and post-transcriptional [2] events have been suggested to mediate the response of GLUT5 expression to fructose. Streptozotocin-induced diabetes has been shown to increase the GLUT5 expression in the small intestine [6]. Thus high blood-glucose concentration or high glucagon/insulin ratio may activate GLUT5 gene expression. Moreover, FK (forskolin), a potent stimulator of adenylate cyclase, activates GLUT5 gene transcription and increases the half-life of GLUT5 mRNA in the Caco-2 cells [7]. GLUT5 mRNA levels depend on cAMP levels in sugar-fed Caco-2 cells [4]. We therefore investigated the transcriptional and post-transcriptional events controlling GLUT5 expression to elucidate the fructose-activated pathway.

The interaction of cytoplasmic proteins with a *cis*-acting element in the UTR (untranslated region) of mRNA is critical to the post-transcriptional control of gene expression. Environmental factors, such as nutrients, hormones and growth factors, affect mRNA stability via several 3′-UTR complexes [8]. The effect of glucose on the mRNA stability depends on the transcript studied. The degradation of GLUT1 mRNA is mediated by 3′-AU-rich elements in glucose-deprived cells [9–14], whereas the degradation of protein kinase C β II mRNA is induced by acute hyperglycaemia [15,16]. In contrast, glucose increases insulin mRNA stability via the binding of proteins to a pyrimidine-rich 3′-UTR sequence [17] and doubles the half-life of glucose-6-phosphatase mRNA [18]. No fructose-induced complexes involving the 3′-UTR sequences modulating the mRNA stability have yet been reported. Fructose may regulate the interaction between GLUT5 mRNA with cytoplasmic protein via the cAMP-signalling pathway. Indeed, cAMP stabilizes several mRNA species [19,20], including, in particular, the transcript of the SGLT1 gene involved in the intestinal uptake of glucose [21,22]. Several 3′-UTR–protein complexes have already been identified. cAMP induces the binding of a 48 kDa complex to a U-rich region in the SGLT1–3′-UTR, resulting in an increase in mRNA stability [23,24]. The cAMP-mediated stabilization of the phosphoenolpyruvate carboxykinase mRNA is correlated with the abundance of 100 kDa protein–3′-UTR complex [25]. The GLUT5 RNA–protein complexes involved in response to fructose stimulation are searched for.

The aim of the present study is to determine how fructose stimulates GLUT5 production more efficiently than glucose, taking into account the contribution of cAMP in this process. We therefore compared the effects of cAMP produced after culture in the presence of fructose, with FK stimulation of the cells. We compared the effects of fructose or glucose in the medium with those of sugar deprivation in Caco-2 cells to assess GLUT5

Abbreviations used: Bt_2, dibutyryl; DAB, 1,4-dideoxy-1,4-imino-arabinitol; DTT, dithiothreitol; FK, forskolin; DDFK, dideoxy-FK; GST, glutathione S-transferase; poly(A)$^+$, polyadenylated; PABP, poly(A)$^+$-binding protein; Paip, PABP-interacting protein; PKA, protein kinase A; UTR, untranslated region.

[1] To whom correspondence should be addressed (e-mail edith.laroche-u505@bhdc.jussieu.fr).

promoter activity and post-transcriptional events affecting mRNA stability and to identify specific mRNA–protein complexes.

EXPERIMENTAL

Cells

Caco-2/PD7 and Caco-2/TC7 cells, two clones derived from the Caco-2 cell line that display high levels of sugar transporter activity, were used in the present study [26]. Both gave similar results. Cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies, Glasgow, Scotland, U.K.), supplemented with 25 mM glucose, 20 % (v/v) heat-inactivated (30 min, 56 °C) foetal calf serum (Abcys, Paris, France), 1 % non-essential amino acids (Life Technologies), 100 units/ml penicillin and 100 μg/ml streptomycin [26]. From day 10 to harvest at day 20, cells were cultured in Dulbecco's modified Eagle's medium and then supplemented with 10 % (v/v) heat-inactivated dialysed serum (dialysis molecular mass cut at 2 kDa), providing hexose-free culture conditions when necessary [4]. Cells were grown on solid support, so that fructose and glucose were delivered only to the apical brush-border membrane of differentiated cells where GLUT5 is expressed. Subsequent biochemical assays were made either immediately after emptying the culture media and keeping it on ice during the experiment, or for cAMP assays by snap-freezing the monolayer by floating the flask on liquid nitrogen.

Chemicals

H89, a specific PKA (protein kinase A) inhibitor and Bt_2 (dibutyryl)-cAMP, a non-metabolizable analogue of cAMP, were obtained from Biomol Research Laboratories (Plymouth Meeting, PA, U.S.A.). The transcription inhibitors 5,6-dichlorobenzimidazole riboside, FK and DDFK (dideoxy-FK), an analogue of FK with some of the effects of FK but not the effect of stimulating adenylate cyclase, were obtained from Calbiochem (La Jolla, CA, U.S.A.). All other chemicals, including DAB (1,4-dideoxy-1,4-imino-arabinitol), were obtained from Sigma–Aldrich (L'Isle-d'Abeau, Chesnes, France). [α-^{32}P]UTP (800 Ci/mmol) was obtained from NEN Life Science Products (Brussels, Belgium).

Biochemical assays

Luciferase assays were performed with the Luciferase Assay System (Promega, Madison, WI, U.S.A.) in a Lumat LB9501 luminometer (Berthold Detection System, Germany). Intracellular cAMP content was determined with the Biotrak cAMP EIA (enzyme immunoassay) system (Amersham Biosciences, Little Chalfont, Bucks., U.K.), according to the manufacturer's instructions. Glycogen assays were performed as described elsewhere [27].

Northern blotting

Total RNA was extracted with the TRI Reagent, according to the manufacturer's instructions (Molecular Research Centre, Cincinnati, OH, U.S.A.). We loaded the equivalent of 15 μg of RNA, in glyoxal-denatured samples, on to 1 % agarose gels and subjected them to electrophoresis. The resulting bands were transferred to membranes (Appligen-Oncor, Ilkirch, France), which were probed with appropriate cDNA radiolabelled with the Megaprime DNA Labelling System (Amersham Biosciences). The membrane and probe were prehybridized and hybridized in 0.25 M phosphate buffer [7 % (w/v) SDS/1 % BSA/1 mM EDTA, pH 6.8] at 65 °C. The membrane was washed under conditions of increasing stringency, ending with a 10 min wash in 0.1 % SDS, 0.1 × SSC (15 mM NaCl/1.5 mM sodium citrate) at 65 °C, and placed against X-ray film.

Vectors

The 5′-deletion constructs were generated from the −2500/+41 region of the GLUT5 promoter inserted into the episomal replicative p205-GTI plasmid. Caco-2/TC7 and Caco-2/PD7 cells were stably transfected with these constructs [28]. Studies on the UTRs of the GLUT5 mRNA were performed with the full-length GLUT5 cDNA. A human small-intestine Marathon-ready cDNA library (ClonTech, NJ, U.S.A.) generated from the poly(A)$^+$ (polyadenylated) RNA of three Caucasian individuals was used for the amplification of the 1–2218 bp human GLUT5 gene by PCR, using sense 5′-CTTCTCTCTCCATTCAGTGCACGCG-3′ primer (position 1–25) and antisense 5′-GAAAGTGATCAGGTT-CATTTTATTGACTAC-3′ primer (position 2189–2218). The full-length GLUT5 cDNA was inserted into pGEM3Z (Promega) for *in vitro* transcription. An *Avr*II restriction site was created by site-directed mutagenesis (Stratagene) at position 1590, at the junction between the open reading frame and the 3′-UTR of the cDNA. The *Avr*II and *Kpn*I sites, located downstream from the GLUT5 cDNA, were used to generate the 3′-UTRG5-pGEM3Z construct for transcription of the GLUT5 3′-UTR with the Riboprobe *In Vitro* Transcription System (Promega).

Interaction of cytosolic protein with GLUT5 3′-UTR RNA

Cytosolic protein extracts (S100) were prepared from cells cultured in sugar-free medium, or in medium containing glucose or fructose. Cells were washed in cold PBS and homogenized in 10 mM Tris (pH 7.4), 10 mM KCl, 1.5 mM magnesium acetate, 1 mM DTT (dithiothreitol), 0.4 mM PMSF, 1 mM sodium orthovanadate, 30 mM β-glycerophosphate and 1 % protease inhibitor cocktail. Homogenates were centrifuged at 14 000 ***g*** for 2 min at 4 °C. The supernatants were collected and centrifuged at 100 000 ***g*** for 1 h at 4 °C to give the S100 supernatants. Aliquots of the S100 supernatants were frozen in liquid nitrogen.

RNA electrophoretic mobility-shift assays were performed with cytosolic S100 (5 μg of protein equivalent), which were incubated for 30 min at 30 °C with 1×10^6 c.p.m. of α-^{32}P-radiolabelled RNA in 10 mM Hepes, 5 mM $MgCl_2$, 50 mM KCl, 0.5 mM EGTA, 0.5 mM DTT, 5 mg/ml heparin, 100 μg/ml tRNA and 10 % (v/v) glycerol. Samples were digested with RNase T1 for 10 min at 30 °C and subjected to electrophoresis on a 7 % (w/v) acrylamide gel. Anti-Paip2 [poly(A)$^+$-binding protein (PABP)-interacting protein 2] polyclonal antibody (9–30 μg) was obtained by immunization of rabbits with a GST (glutathione S-transferase)–Paip2 fusion protein (Eurogentec, Angers, France). Antisera were depleted of anti-GST antibodies and purified on a GST–Paip2/epoxy-aminohexyl–Sepharose 4B affinity column. The anti-Paip2 antiserum was incubated with the [^{32}P]-3′-UTR RNA for 30 min at 30 °C. Its effects were identical regardless of whether it was added with the reaction mixture before addition of the 3′-UTR probe or after formation of the complex.

For UV cross-linking, we incubated 50 μg of cytosolic protein for 30 min at 25 °C, with 1×10^6 c.p.m. of α-^{32}P-radiolabelled RNA in 10 mM Hepes buffer [3 mM $MgCl_2$/40 mM KCl/5 % glycerol/1 mM DTT]. We added heparin (5 mg/ml) and exposed the samples to light with energy density 2.5 J/cm^2, at a wavelength of 312 nm (Biosun RMX3W, Vilber Lourmat, Marnes la Vallée, France). Naked RNA was digested with 1 mg/ml RNase I and

5 units of RNase TI for 30 min at 37 °C. Protein–RNA complexes were separated by electrophoresis on a 10 % acrylamide gel containing SDS after denaturation of the sample by heating for 5 min at 65 °C in 5 % glycerol, 1 % SDS, 0.04 M Tris (pH 6.8), 0.05 M DTT and Bromophenol Blue. The gel was then vacuum-dried and placed against X-ray film.

For Northwestern analysis, we treated cytosolic S100 extracts (50 μg) as described for UV cross-linking, subjected them to electrophoresis on a gel containing 10 % acrylamide and 0.1 % SDS and transferred the resulting bands to Hybond nitrocellulose membranes in a buffer consisting of 250 mM Tris, 0.1 % SDS and 300 mM glycine. The protein bands were renatured, and the membrane was probed with 300 c.p.m./ml α-^{32}P-labelled RNA [29]. The blots were then incubated with anti-Paip2 antibodies (1/2000) and antibody binding was detected by enhanced chemiluminescence (ECL®), according to the manufacturer's instructions (Amersham Biosciences).

RESULTS

Stimulation of GLUT5 expression by fructose, glucose and cAMP

We assessed GLUT5 expression in differentiated Caco-2 cells by determining the GLUT5 mRNA levels, promoter activity and intracellular cAMP concentration. GLUT5 mRNA levels were 2.5 times higher in fructose-fed cells when compared with glucose-fed cells, whereas in sugar-deprived cells, we were not able to detect GLUT5 mRNA (Figure 1A, left panel). Surprisingly, in these cells, both fructose and glucose induced GLUT5 promoter activity by a factor of 5 with respect to sugar-deprived cells (Figure 1C, left panel). Cellular cAMP concentration, which was determined in parallel, was significantly higher ($P < 0.05$) in cells cultured in the presence of fructose when compared with those cultured in the presence of glucose, with cAMP concentration in the presence of fructose being twice that in sugar-deprived cells (Figure 1B, left panel). Thus fructose and glucose stimulated GLUT5 promoter activity to similar extents, despite differences in cellular cAMP concentration.

FK treatment had various effects on cAMP concentration. DDFK, an FK analogue that does not stimulate adenylate cyclase, was used as a control. At FK concentrations of 250 nM and 25 μM, GLUT5 mRNA levels were three and 6.5 times higher respectively when compared with those for the untreated control or DDFK-treated cells (Figure 1A, right panel). GLUT5 mRNA and cAMP levels (Figure 1B, right panel) remained constant in the presence of FK, up to a concentration of 2.5 nM. A concentration of 250 nM FK increased cAMP concentration by a factor of 1.8 and GLUT5 promoter activity by a factor of 1.5, whereas 25 μM FK increased cAMP concentration by a factor of 25 and GLUT5 promoter activity by a factor of 2.2 (Figure 1C, right panel). Thus GLUT5 mRNA synthesis and promoter activity were stimulated by FK in a dose-dependent manner, which at the same time increased the cAMP concentration in the cells.

We then performed experiments to localize fructose- and glucose-responsive elements within the GLUT5 promoter (Figure 2) using 5′-deletion constructs that were designed to delete successively the two putative cAMP-response elements identified in the sequence of the GLUT5 promoter at positions −365/−358 and −332/−325 respectively from start of transcription. We measured the activity of various 5′-deleted GLUT5 promoter constructs in cells cultured with fructose, glucose and in the absence of any sugar. Fructose and glucose stimulated the activity of all constructs derived from the GLUT5 promoter, by a factor of 5–6 in each case with respect to sugar-free culture conditions (Figure 2, lower panel). Thus the two sugars increased the GLUT5 promoter activity to similar, extents and the −272/+41 GLUT5 promoter is sufficient for the sugar response.

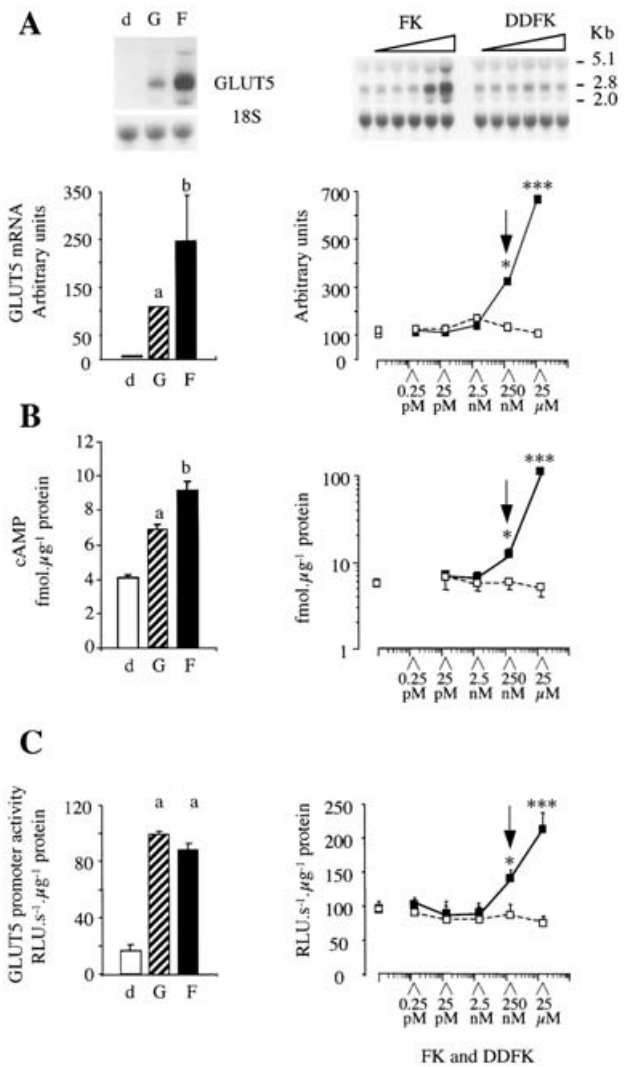

Figure 1 Increases in GLUT5 mRNA levels and promoter activity stimulated by FK or sugar

Caco-2 cells were stably transfected with the −2500/+41 promoter region of the GLUT5 gene fused to the luciferase reporter gene. Caco-2 cells were cultured for 10 days (left panels) in the absence of sugar (d, white bars) or in the presence of 25 mM glucose (G, hatched bars) or fructose (F, black bars). Statistical differences are indicated by lower-case letters: a and b denote statistical difference ($P < 0.001$) between glucose-fed and sugar-deprived cells, and between fructose and glucose culture conditions respectively. Cells cultured with glucose were treated with various concentrations (0, 0.25 pM, 25 pM, 2.5 nM, 250 nM, 25 μM) of FK (■) or DDFK (□) for 48 h (right panels). (**A**) Northern-blot analysis of total RNA and quantification by densitometry. (**B**) cAMP content of the various cells. (**C**) GLUT5 promoter activity, assayed by measuring luciferase activity. Results are means of duplicate determinations for 2–4 independent cultures. Densitometric analysis and luciferase activities are expressed as a percentage of the values obtained for untreated glucose controls. Statistical differences between FK and DDFK results are indicated by $^{*}P < 0.05$ and $^{***}P < 0.001$ respectively. Note that 250 nM FK and 25 mM fructose gave similar increases in GLUT5 mRNA and cAMP levels (**A**, **B**), whereas only FK (not fructose) increased GLUT5 promoter activity (**C**). RLU, relative light units.

Overall, the results indicate that the greater increase in endogenous GLUT5 mRNA levels in cells cultured in the presence of fructose compared with the cells cultured in the presence of glucose was not due to a higher rate of transcription, despite the difference in cellular cAMP concentration.

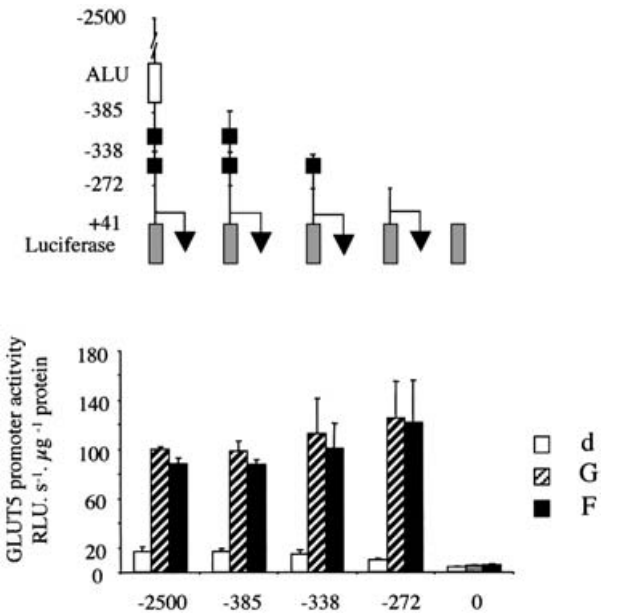

Figure 2 Effect of 5′-deletions of the GLUT5 promoter on luciferase activity under various sugar culture conditions

Upper panel: schematic representation of the 5′-deletions of the GLUT5 promoter, driving the luciferase reporter gene with the solid squares, showing the position of two putative cAMP-response elements identified in the sequence of the human promoter at position −365/−358 and −332/−325 from start of transcription. Lower panel: luciferase activities were measured in cells cultured for 10 days in the absence of sugar (d, white bars), or in the presence of 25 mM glucose (G, hatched bars) or fructose (F, black bars). There was a significant difference between activities in sugar-fed cells ($P < 0.001$) compared with sugar-deprived (d, open bars) controls. RLU, relative light units.

Sugar metabolism and PKA signalling mediate the stimulation of GLUT5 transcription

Increasing the cAMP content in Caco-2 cells rapidly induces glycogen store mobilization and it also increases the rate of glucose consumption [30]. We therefore hypothesized that cAMP (FK) stimulated promoter activity by inducing sugar metabolism. We investigated whether this was indeed the case by evaluating glycogen stores and promoter activities in the −2500/+41 construct as a function of sugar and FK (50 μM) treatment. Glycogen stores were similar in the presence of fructose and glucose, and were 1.6 times larger in cells cultured in the presence of one of these sugars compared with sugar-deprived cells (Figure 3A). FK treatment reduced glycogen stores by 40% in cells cultured in media containing fructose or glucose, to levels similar to those observed in the absence of sugar (Figure 3A). Luciferase activity was five times higher in the presence of sugar than in its absence (Figure 3B). GLUT5 promoter activity was stimulated by FK even in the absence of sugar.

To investigate the role of glycogen metabolism in the regulation of GLUT5 promoter activity, we used a potent inhibitor of glycogenolysis, targeting glycogen phosphorylase activity. This inhibitor DAB was added to the culture medium at a concentration of 50 μM and the cells were incubated in this medium for 24 h [31]. In control cells, DAB did not affect basal promoter activity (Figure 3C). Bt_2-cAMP (500 μM), a cAMP analogue was also used, as it is a less potent activator of the cAMP transduction pathway. Bt_2-cAMP increased the activity of the −2500/+41 GLUT5 promoter by a factor of 1.5. DAB reduced the effect of Bt_2-cAMP by 60%. We then used H89 (50 μM), an inhibitor of PKA [32]. H89 totally prevented GLUT5 promoter activation by

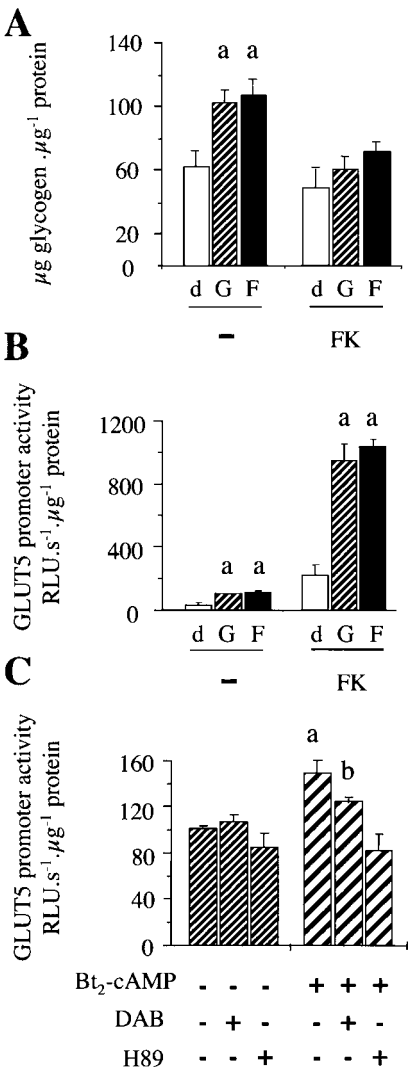

Figure 3 Involvement of glycogen metabolism and PKA pathway in the regulation of GLUT5 transcription

(**A**) Effect of FK on the glycogen content of cells cultured in the presence of sugars. Results are expressed as μg of glycogen/μg of protein (means ± S.E.M. of duplicate determinations for five independent experiments). The letter 'a' denotes a significant difference ($P < 0.005$) in comparison of cells cultured in the presence of glucose (G, hatched bars) or fructose (F, black bars) and absence of sugar (d, empty bars). (**B**) Effect of FK on GLUT5 (2500/+41) promoter activity in cells cultured as a function of sugar in the culture medium. The letter 'a' denotes a significant difference ($P < 0.005$) between FK-treated and the corresponding untreated (−) cells. (**C**) GLUT5 promoter activity was measured in glucose-fed cells (hatched bars) in the presence or absence of 0.5 mM Bt_2-cAMP treatment (dark hatched bars) and further treated with the inhibitor of glycogenolysis (0.1 mM DAB) or the inhibitor of PKA (0.05 mM H89). Lower-case letters denote significant differences: a, $P < 0.005$ and b, $P < 0.05$ from control cells. RLU, relative light units.

Bt_2-cAMP (Figure 3C). We therefore concluded that the activation of the GLUT5 promoter by cAMP is mediated entirely by PKA signalling, with at least half the effect due to glucose generated from glycogen degradation.

Correlation between the half-life of GLUT5 mRNA and cellular cAMP content

We then investigated the effect of cAMP-pathway inhibitors on endogenous GLUT5 mRNA abundance (Figure 4A). Bt_2-cAMP increased mRNA levels by a factor of 2.7 with respect to controls.

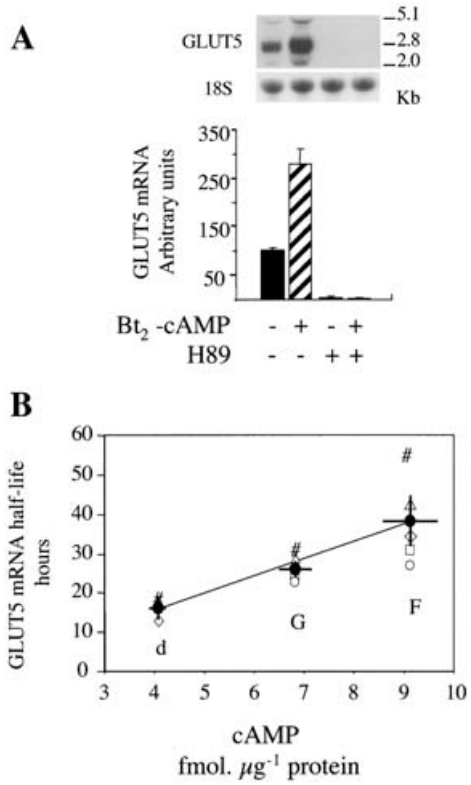

Figure 4 The cAMP pathway and half-life of GLUT5 mRNA

(**A**) Caco-2/TC7 cells cultured with fructose (black bars) were treated for 24 h with 0.5 mM Bt_2-cAMP (dark hatched bars) in the presence or absence of 0.05 mM H89. Endogenous GLUT5 mRNA levels and densitometric analysis are shown. (**B**) The half-life of GLUT5 mRNA was measured in Caco-2/TC7 cells treated with two transcription inhibitors, 0.2 M 5,6-dichloro-benzimidazole riboside or 5 μg/μl actinomycin D, with identical results. mRNA levels were quantified by densitometric analysis of Northern blots, and half-lives were plotted as a function of cAMP content for cells grown in the absence of sugar (d) or in the presence of 25 mM glucose (G) or fructose (F). Various symbols have been used to highlight measurements that have been made in parallel. The bold line is the regression curve for all experiments ($R^2 = 0.9913$).

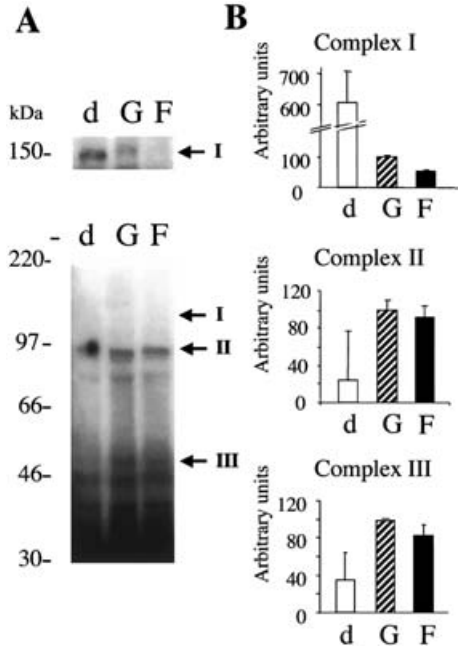

Figure 5 UV cross-linking of GLUT5 3′-UTR with cytosolic protein extracts

Left panel represents the UV cross-linking of radiolabelled GLUT5 3′-UTR with 50 μg of cytosolic protein extract of cells cultured under various sugar conditions. Cell protein extracts were prepared from cells grown in the absence of sugar (d, open bars), or in the presence of 25 mM glucose (G, hatched bars) or fructose (F, black bars). Three main complexes were formed: a 140 kDa complex (complex I) detected only after exposure for 1 week (upper left panel), and the 96 kDa complex II and the 50 kDa complex III, which were detected after 24 h. Right panel shows the densitometric analysis of 2–4 independent experiments. Note that the amount of complex I decreases in the presence of sugar, whereas the amounts of complexes II and III increase.

The inhibition of glycogenolysis by DAB did not affect GLUT5 mRNA levels (results not shown). In contrast, H89 dramatically reduced (95%) both the control and cAMP-stimulated GLUT5 mRNA levels. Similar results were obtained with cells cultured in the presence of fructose (Figure 4A) and those cultured in the presence of glucose (results not shown). Thus PKA inhibition by H89 prevented GLUT5 mRNA accumulation continuing GLUT5 promoter activity, as shown in Figure 3. This strongly suggests that cAMP is involved in the regulation of the half-life of GLUT5 mRNA.

We measured the half-life of GLUT5 mRNA in transcription-arrested cells cultured in medium containing glucose or fructose or in sugar-free medium. GLUT5 mRNA was barely detectable in cells subjected to chronic sugar deprivation (Figure 1A), due to inactivation of the GLUT5 promoter (Figure 2B). We determined the half-life of GLUT5 mRNA in sugar-deprived cells under conditions of simultaneous glucose withdrawal and transcription inhibitor addition. The half-life of GLUT5 mRNA was determined from mRNA decay curves (results not shown) and plotted against the cAMP content of the cells (Figure 4B). The half-life of GLUT5 mRNA increased from 17 h in sugar-deprived cells to 24 and 36 h in cells cultured in the presence of glucose and fructose respectively. The half-life of GLUT5 mRNA was linearly correlated with cAMP concentration ($R^2 = 0.9913$). Thus both fructose and cAMP seem to exert their effects on GLUT5 production by increasing GLUT5 mRNA stability.

Sugar-dependent binding of cytosolic proteins to GLUT5 3′-UTR RNA

Regulatory interaction often involves the 3′-UTR of mRNA [33] and modulates processing. To elucidate the mechanism by which fructose regulates GLUT5 production, we analysed the binding of cytosolic proteins to the GLUT5 (1590–2218) 3′-UTR. 3′-UTR RNA was UV-cross-linked in the presence of cytosolic S100 protein extracts from cells cultured in the presence or absence of fructose and glucose (Figure 5). Three main RNA–protein complexes were obtained, which we identified as complexes I, II and III, on the basis of molecular mass.

The abundance of complex I (140 kDa; Figure 5A) decreased under conditions of increased GLUT5 mRNA abundance. The binding of complex I was 12 and six times higher in sugar-deprived cells than in cells grown in the presence of fructose and glucose respectively (Figure 5B). Complex I may thus be involved in GLUT5 mRNA degradation in sugar-deprived cells and may contribute to the difference in mRNA stability in cells cultured in the presence of fructose and glucose.

Complexes II and III were more abundant in cytosolic protein extracts from cells cultured in the presence of fructose or glucose when compared with that of sugar-deprived cells. These

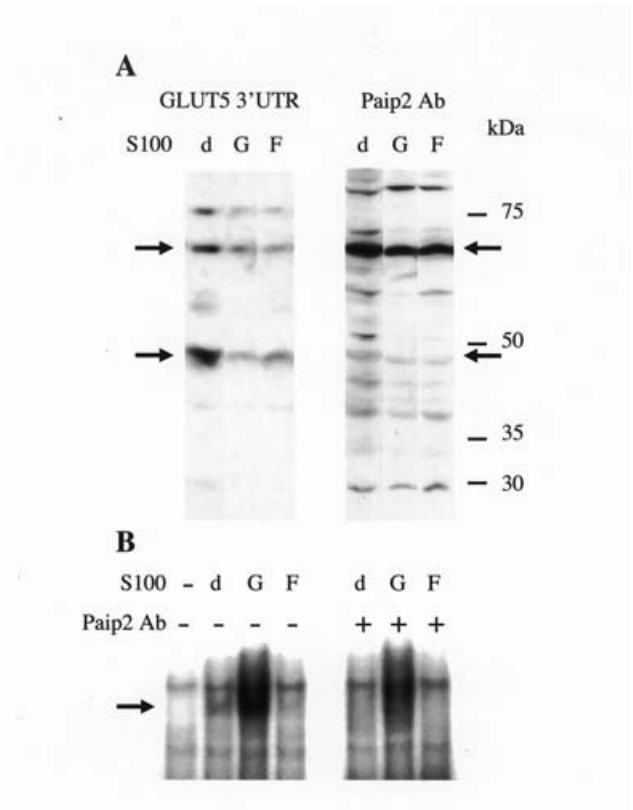

Figure 6 Northwestern and Western-blot analyses of RNA-binding proteins (Paip2 is a partner of the GLUT5 RNA–protein complex)

(**A**) Cytosolic protein (50 μg) extracted from cells cultured in the absence of sugar (d) or in the presence of glucose (G) or fructose (F) was resolved by electrophoresis after mild denaturation. The protein bands were transferred to a membrane, which was probed with the radiolabelled GLUT5 3′-UTR for Northwestern analysis (left panel) or an anti-Paip2 antibody for Western-blot analysis (upper right panel). Note the presence of two bands, indicated by arrows, 68 and 48 kDa in mass, that bound both the anti-Paip2 antibody and the radiolabelled probe. (**B**) Representative blot with the detection of a radiolabelled GLUT5–3′-UTR complex with 5 μg of cytosolic proteins from cells cultured under various sugar conditions. Incubations with the anti-Paip2 antibody before (right panel 3 lanes) and after (results not shown) complex formation gave similar results. Note the disappearance of the complex (arrow) in the presence of the anti-Paip2 antibody.

complexes had molecular masses of 96 and 48 kDa respectively. Densitometric analysis (Figure 5B) showed that the binding activity of these complexes was 3–5 times higher in glucose or fructose when compared with sugar-deprived cell extracts. These complexes may protect GLUT5 mRNA from degradation in sugar-fed cells, but do not discriminate between fructose and glucose.

GLUT5 mRNA 3′-UTR–protein complexes contain Paip2

To identify the proteins that bind GLUT5 3′-UTR, we performed Northwestern blotting with cytosolic proteins from cells grown in the presence of fructose or glucose and the cells grown in the absence of sugar. Three protein bands, 85, 68 and 48 kDa in mass, were labelled with the GLUT5 3′-UTR probe (Figure 6A, left panel), and found in more abundance in sugar-deprived cell extracts when compared with extracts of cells cultured in the presence of glucose or fructose. This sugar relationship is similar to that of complex I identified by UV cross-link (Figure 5).

Paip2, a partner of the PABP, is thought to repress translation [34,35]. Anti-Paip2 antibodies were used to investigate the possible role of this protein in the regulation of GLUT5 mRNA degradation (Figure 6A, right panel). On Western blots, we detected a 28 kDa protein of the same mass as of native Paip2, the amount of which did not depend on the culture conditions. Two other bands at 68 and 48 kDa, which corresponded to proteins less abundant in the presence of fructose or glucose than in sugar-deprived extracts, were recognized by the anti-Paip2 antibody, and by GLUT5 3′-UTR hybridization (Figure 6A). We suspect that the conditions used to denature cytosolic proteins in the extracts may not have disrupted some strong protein interactions. These results therefore suggest that Paip2 is a component of at least one complex binding to GLUT5 3′-UTR RNA.

We then assessed the direct contribution of Paip2 to a complex interacting with GLUT5 3′-UTR (Figure 6B). RNA electrophoretic mobility-shift assay profiles revealed a major RNase T1-resistant complex bound to the radiolabelled 3′-UTR of GLUT5. Strong binding activity was observed in extracts of cells cultured with glucose, and lower levels of binding were observed in extracts of cells grown with fructose or in the absence of sugar. The anti-Paip2 antibody prevented the formation of this complex in all cell extracts used. This lack of RNA supershift suggests that the polyclonal antibody interferes with RNA recognition and disrupts GLUT5–protein complex formation. Thus Paip2 is a component of the protein complex binding the GLUT5 mRNA.

DISCUSSION

In the present study, we investigated the underlying mechanisms responsible for the up-regulation of GLUT5 production (*SLC2A5* gene) by fructose and glucose. Fructose gave more stable GLUT5 transcripts than glucose; this difference may be responsible for specific fructose-induced GLUT5 expression.

We were able to dissect the sugar response of the GLUT5 promoter because it was possible to manipulate the content of the extracellular medium with the Caco-2 cell culture. Fructose and glucose were found to stimulate the GLUT5 promoter activity similarly in Caco-2 cells. This finding conflicts with previous observations of the transcriptional effects of fructose and glucose made with nuclei from mucosal scrapings of rat jejunum [5]. This difference in results may be due to differences in the cells used: a fully differentiated Caco-2 enterocyte sample in the present study, and a heterogeneous intestinal sample containing proliferating cells and cells at various stages of differentiation in the previous study. Consistent with this hypothesis, glucose regulation of *SGLT1* gene expression starts only at the crypt–villus junction [36]. Thus comparison of the results obtained *in vivo* and with Caco-2 cells may reveal differentiation-related regulation of GLUT5 production by fructose.

The stimulation of GLUT5 mRNA synthesis by fructose is more than a simple effect on transcription. All promoter constructs were stimulated to a similar extent by fructose and glucose, suggesting that there is a sugar-response element in the first 272 bp of GLUT5 promoter. Activation of the cAMP pathway by FK increased the activity of the 2500 bp GLUT5 promoter, but only half of the effect was obtained with the approx. 272 bp [7] containing a sugar-response element. This may be due to the activation of sugar metabolism in Caco-2 cells by FK [30], as shown by the results obtained with inhibitors of glycogen degradation or of PKA activity.

Although fructose and FK had similar effects on cAMP levels, FK had a larger effect than fructose on GLUT5 gene transcription. As FK is lipophilic and a very potent activator of

adenylate cyclase, it has been suggested that its effects may spread through the cell, triggering transcription in a more sustained way. Furthermore, the local activation of adenylate cyclase in the vicinity of the plasma membrane has been demonstrated in human cells [37–39]. Thus fructose transport is likely to trigger a local increase in cAMP levels.

Comparison of the effects of fructose and glucose suggests that the fine regulation of GLUT5 production by fructose is modulated at the post-transcriptional level. Several lines of evidence support this hypothesis. First, transcription did not account for differences in GLUT5 mRNA levels in the presence of glucose and fructose. Secondly, an inhibitor of PKA destabilized GLUT5 mRNA, without affecting basal levels of transcription. Thirdly, the half-life of GLUT5 mRNA and cellular cAMP levels were strictly correlated. We identified several RNA–protein complexes (140, 96 and 48 kDa) that displayed differential binding to the GLUT5 3′-UTR depending on sugar conditions. A 140 kDa complex I was most abundant when levels of GLUT5 mRNA degradation were high, suggesting that this complex may be a destabilizing complex. The levels of this complex were lower in the presence of glucose, and even lower in the presence of fructose, when cAMP content increased. The abundance of complex I was lowest with cytosolic extracts from cells cultured with fructose. Therefore, this complex may be involved in the differential simulation of GLUT5 expression by fructose and glucose. However, formation of the 96 and 48 kDa complexes was strongly induced by glucose and fructose, and the binding of these two complexes was similar in the presence of glucose and fructose, indicating a general sugar effect. A 48 kDa complex that bound to a U-rich region in the 3′-UTR of the SGLT1 transcript has also been identified, and the amount of this complex increases with isobutylmethylxanthine treatment of the cells, suggesting the involvement of a cAMP-regulated process [21,22]. Integrated regulation of the expression of transporters in enterocytes by fructose and cAMP involves the processing of mRNA through several complexes, the partners of which remain to be identified.

Paip2 has been reported to act as a translational repressor, interacting with PABP, which disrupts the circularization of mRNA and inhibits translation. Paip2 is resident in the cytosol and may interact with the 3′-UTR of several mRNAs. The anti-Paip2 antibody recognized a protein complex bound to the GLUT5 3′-UTR in glucose-deprived cells in which GLUT5 is produced only in small amounts. It has been suggested that GLUT5 production is regulated translationally in the small intestine of the rat [2]. Consistent with this, Paip2 was essentially associated with low levels of GLUT5 production and low levels of mRNA stability, suggesting that the binding of this protein to the GLUT5 3′-UTR destabilizes transcripts.

Overall, our results show that fructose stimulates the expression of GLUT5 by increasing both the rate of transcription of the GLUT5 gene and mRNA stability. Both fructose and glucose have similar effects on the transcription of the gene, suggesting that a common metabolite of these two molecules is involved. The differences in the effects of fructose and glucose on mRNA degradation are probably due to differences in the capacities of the two sugars to increase the cAMP levels locally and to modulate the formation of protein complexes with GLUT5 3′-UTR. Paip2 was identified as one of the partners in these complexes.

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REFERENCES

1 Kayano, T., Burant, C. F., Fukumoto, H., Gould, G. W., Fan, Y. S., Eddy, R. L., Byers, M. G., Shows, T. B., Seino, S. and Bell, G. I. (1990) Human facilitative glucose transporters. Isolation, functional characterization, and gene localization of cDNAs encoding an isoform (GLUT5) expressed in small intestine, kidney, muscle, and adipose tissue and an unusual glucose transporter pseudogene-like sequence (GLUT6). J. Biol. Chem. **265**, 13276–13282

2 Burant, C. F. and Saxena, M. (1994) Rapid reversible substrate regulation of fructose transporter expression in rat small intestine and kidney. Am. J. Physiol. **267**, G71–G79

3 Corpe, C. P., Bovelander, F. J., Munoz, C. M., Hoekstra, J. H., Simpson, I. A., Kwon, O., Levine, M. and Burant, C. F. (2002) Cloning and functional characterization of the mouse fructose transporter, GLUT5. Biochim. Biophys. Acta **1576**, 191–197

4 Mesonero, J., Matosin, M., Cambier, D., Rodriguez-Yoldi, M. J. and Brot-Laroche, E. (1995) Sugar-dependent expression of the fructose transporter GLUT5 in Caco-2 cells. Biochem. J. **312**, 757–762

5 Kishi, K., Tanaka, T., Igawa, M., Takase, S. and Goda, T. (1999) Sucrase–isomaltase and hexose transporter gene expressions are coordinately enhanced by dietary fructose in rat jejunum. J. Nutr. **129**, 953–956

6 Burant, C. F., Flink, S., DePaoli, A. M., Chen, J., Lee, W. S., Hediger, M. A., Buse, J. B. and Chang, E. B. (1994) Small intestine hexose transport in experimental diabetes. Increased transporter mRNA and protein expression in enterocytes. J. Clin. Invest. **93**, 578–585

7 Mahraoui, L., Takeda, J., Mesonero, J., Chantret, I., Dussaulx, E., Bell, G. I. and Brot-Laroche, E. (1994) Regulation of expression of the human fructose transporter (GLUT5) by cyclic AMP. Biochem. J. **301**, 169–175

8 Ross, J. (1996) Control of messenger RNA stability in higher eukaryotes. Trends Genet. **12**, 171–175

9 Dwyer, K. J., Boado, R. J. and Pardridge, W. M. (1996) *Cis*-element/cytoplasmic protein interaction within the 3′-untranslated region of the GLUT1 glucose transporter mRNA. J. Neurochem. **66**, 449–458

10 Boado, R. J. and Pardridge, W. M. (1998) Ten nucleotide *cis* element in the 3′-untranslated region of the GLUT1 glucose transporter mRNA increases gene expression via mRNA stabilization. Brain Res. Mol. Brain Res. **59**, 109–113

11 Boado, R. J. and Pardridge, W. M. (2002) Glucose deprivation and hypoxia increase the expression of the GLUT1 glucose transporter via a specific mRNA *cis*-acting regulatory element. J. Neurochem. **80**, 552–554

12 Boado, R. J. and Pardridge, W. M. (1993) Glucose deprivation causes posttranscriptional enhancement of brain capillary endothelial glucose transporter gene expression via GLUT1 mRNA stabilization. J. Neurochem. **60**, 2290–2296

13 Kumagai, A. K., Kang, Y. S., Boado, R. J. and Pardridge, W. M. (1995) Upregulation of blood–brain barrier GLUT1 glucose transporter protein and mRNA in experimental chronic hypoglycemia. Diabetes **44**, 1399–1404

14 Hamilton, B. J., Nichols, R. C., Tsukamoto, H., Boado, R. J., Pardridge, W. M. and Rigby, W. F. (1999) hnRNP A2 and hnRNP L bind the 3′UTR of glucose transporter 1 mRNA and exist as a complex *in vivo*. Biochem. Biophys. Res. Commun. **261**, 646–651

15 Patel, N. A., Chalfant, C. E., Yamamoto, M., Watson, J. E., Eichler, D. C. and Cooper, D. R. (1999) Acute hyperglycemia regulates transcription and post-transcriptional stability of PKCβII mRNA in vascular smooth muscle cells. FASEB J. **13**, 103–113

16 Patel, N. A., Yamamoto, M., Illingworth, P., Mancu, D., Mebert, K., Chappell, D. S., Watson, J. E. and Cooper, D. R. (2002) Phosphoinositide 3-kinase mediates protein kinase C β II mRNA destabilization in rat A10 smooth muscle cell cultures exposed to high glucose. Arch. Biochem. Biophys. **403**, 111–120

17 Tillmar, L., Carlsson, C. and Welsh, N. (2002) Control of insulin mRNA stability in rat pancreatic islets. Regulatory role of a 3′-untranslated region pyrimidine-rich sequence. J. Biol. Chem. **277**, 1099–1106

18 Massillon, D. (2001) Regulation of the glucose-6-phosphatase gene by glucose occurs by transcriptional and post-transcriptional mechanisms. Differential effect of glucose and xylitol. J. Biol. Chem. **276**, 4055–4062

19 Erondu, N. E., Dake, B. L., Moser, D. R., Lin, M., Boes, M. and Bar, R. S. (1996) Regulation of endothelial IGFBP-3 synthesis and secretion by IGF-I and TGF-β. Growth Regul. **6**, 1–9

20 Tillmann-Bogush, M., Heaton, J. H. and Gelehrter, T. D. (1999) Cyclic nucleotide regulation of PAI-1 mRNA stability. Identification of cytosolic proteins that interact with an A-rich sequence. J. Biol. Chem. **274**, 1172–1179

21 Peng, H. and Lever, J. E. (1995) Regulation of Na^+-coupled glucose transport in LLC-PK1 cells. Message stabilization induced by cyclic AMP elevation is accompanied by binding of a $M(r) = 48{,}000$ protein to a uridine-rich domain in the 3′-untranslated region. J. Biol. Chem. **270**, 23996–24003

22 Peng, H. and Lever, J. E. (1995) Post-transcriptional regulation of Na^+/glucose cotransporter (SGTL1) gene expression in LLC-PK1 cells. Increased message stability after cyclic AMP elevation or differentiation inducer treatment. J. Biol. Chem. **270**, 20536–20542
23 Loflin, P. and Lever, J. E. (2001) HuR binds a cyclic nucleotide-dependent, stabilizing domain in the 3′ untranslated region of Na^+/glucose cotransporter (SGLT1) mRNA. FEBS Lett. **509**, 267–271
24 Loflin, P. and Lever, J. E. (2001) A *cis*-dominant cyclic nucleotide-dependent regulatory domain in the 3′-untranslated region of Na^+/glucose cotransporter (SGLT1) mRNA. FEBS Lett. **492**, 233–237
25 Nachaliel, N., Jain, D. and Hod, Y. (1993) A cAMP-regulated RNA-binding protein that interacts with phosphoenolpyruvate carboxykinase (GTP) mRNA. J. Biol. Chem. **268**, 24203–24209
26 Mahraoui, L., Rodolosse, A., Barbat, A., Dussaulx, E., Zweibaum, A., Rousset, M. and Brot-Laroche, E. (1994) Presence and differential expression of SGLT1, GLUT1, GLUT2, GLUT3 and GLUT5 hexose-transporter mRNAs in Caco-2 cell clones in relation to cell growth and glucose consumption. Biochem. J. **298**, 629–633
27 Rousset, M., Chevalier, G., Rousset, J. P., Dussaulx, E. and Zweibaum, A. (1979) Presence and cell growth-related variations of glycogen in human colorectal adenocarcinoma cell lines in culture. Cancer Res. **39**, 531–534
28 Matosin-Matekalo, M., Mesonero, J. E., Laroche, T. J., Lacasa, M. and Brot-Laroche, E. (1999) Glucose and thyroid hormone co-regulate the expression of the intestinal fructose transporter GLUT5. Biochem. J. **339**, 233–239
29 Stefanovic, B., Hellerbrand, C., Holcik, M., Briendl, M., Aliebhaber, S. and Brenner, D. A. (1997) Post-transcriptional regulation of collagen α1(I) mRNA in hepatic stellate cells. Mol. Cell. Biol. **17**, 5201–5209
30 Rousset, M., Laburthe, M., Pinto, M., Chevalier, G., Rouyer-Fessard, C., Dussaulx, E., Trugnan, G., Boige, N., Brun, J. L. and Zweibaum, A. (1985) Enterocytic differentiation and glucose utilization in the human colon tumor cell line Caco-2: modulation by forskolin. J. Cell. Physiol. **123**, 377–385

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31 Andersen, B., Rassov, A., Westergaard, N. and Lundgren, K. (1999) Inhibition of glycogenolysis in primary rat hepatocytes by 1,4-dideoxy-1,4-imino-D-arabinitol. Biochem. J. **342**, 545–550
32 Chijiwa, T., Mishima, A., Hagiwara, M., Sano, M., Hayashi, K., Inoue, T., Naito, K., Toshioka, T. and Hidaka, H. (1990) Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, N-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D pheochromocytoma cells. J. Biol. Chem. **265**, 5267–5272
33 Day, D. A. and Tuite, M. F. (1998) Post-transcriptional gene regulatory mechanisms in eukaryotes: an overview. J. Endocrinol. **157**, 361–371
34 Khaleghpour, K., Kahvejian, A., De Crescenzo, G., Roy, G., Svitkin, Y. V., Imataka, H., O'Connor-McCourt, M. and Sonenberg, N. (2001) Dual interactions of the translational repressor Paip2 with poly(A) binding protein. Mol. Cell. Biol. **21**, 5200–5213
35 Khaleghpour, K., Svitkin, Y. V., Craig, A. W., DeMaria, C. T., Deo, R. C., Burley, S. K. and Sonenberg, N. (2001) Translational repression by a novel partner of human poly(A) binding protein, Paip2. Mol. Cell **7**, 205–216
36 Shirazi-Beechey, S. P., Gribble, S. M., Wood, I. S., Tarpey, P. S., Beechey, R. B., Dyer, J., Scott, D. and Barker, P. J. (1994) Dietary regulation of the intestinal sodium-dependent glucose cotransporter (SGLT1). Biochem. Soc. Trans. **22**, 655–658
37 Rich, T. C., Fagan, K. A., Tse, T. E., Schaack, J., Cooper, D. M. and Karpen, J. W. (2001) A uniform extracellular stimulus triggers distinct cAMP signals in different compartments of a simple cell. Proc. Natl. Acad. Sci. U.S.A. **98**, 13049–13054
38 Rich, T. C., Tse, T. E., Rohan, J. G., Schaack, J. and Karpen, J. W. (2001) *In vivo* assessment of local phosphodiesterase activity using tailored cyclic nucleotide-gated channels as cAMP sensors. J. Gen. Physiol. **118**, 63–78
39 Schwartz, J. H. (2001) The many dimensions of cAMP signaling. Proc. Natl. Acad. Sci. U.S.A. **98**, 13482–13484